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Isolation and identification of some fungi and yeasts from the soils of Kirkuk oil fields

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Abstract
The present study included isolating and diagnosing filamentous fungi and yeasts in three months starting from 15/6/2022 to 15/9/2022 from the soils of Bay Hassan oil field in Kirkuk Governorate and the K1 oil filling station in Erbil Governorate/ Iraq. 80 isolates of filamentous fungi were obtained and distributed to 3 different genera *Aspergillus*, *Penicillium* and *Rhizoctonia*. The fungi *A. niger*, *A. caespitosus*, *A. fumigatus*, *A. nidulans*, *A. parasiticus*, *A. emericella quadrilieata* were identified to the species level and the fungus was *A. niger* was the most frequent fungus in the two isolation sites, the number of isolates in (k1) filling station reached 9 (56.25%) and 15 (23.43%) isolates in Bay Hassan field, while the fungus *A. fumigatus* was the least frequent (6.25%) in K1 filling station. *Rhizoctonia* spp. was less frequent in Bay Hassan field (1.56%), and the rest of the fungi varied in their appearance rates. The study was also Isolated62 isolation back to Eight local yeast isolates were isolated, which included eight different types of yeasts: *Pichia*, *Saccharomyces*, *Geotrichum*, *Zygosaccharomyces*, *Kloeckera*, *kazachstania*, *Rhodotorula*, and *Candida*.

Keywords: isolation and identification, included isolating and diagnosing filamentous, Kirkuk oil fields

Introduction
Soil is exposed to pollution due to the accumulation of heavy metals and minerals through emissions from rapidly expanding industrial areas, mine residues, disposal of metal waste, leaded gasoline and paints, the use of fertilizers, pesticides and highly toxic phenolic compounds due to irrigation with sewage water and the possibility of these dangerous pollutants reaching humans through a chain food (Tork *et al*., 2020), and this makes it a suitable environment for the growth and reproduction of many types of organisms, which include bacteria, viruses, protozoa, algae and fungi, including yeasts (Prescott *et al*., 2005), yeasts are found at a depth of 6-10 cm from the surface of the soil and its presence increases more than it is on the upper surface, and its vertical distribution depends on several factors, including pressure, precipitation, cultivation, hiding animals and insects that live in the soil. Increase its ability to withstand low humidity (El-Tarabily and Sivasithamparam, 2006). Yurkov mentioned that basidian yeasts are found more abundantly in soil samples than cystic yeasts, including *Rhodotorula* sp., *Sporolomyces* sp., *Cytobasidium* sp., *Rhodosporidiobolus* sp. and *Vishniacozyma* spp. (Abu-Mejdad *et al*., 2019) [1]. Abu Al-Ghaith and Zaait (2020) [15] were able to isolate some types of fungi, *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *A. nidulans*, *Rhizopus* sp. From soils contaminated with hydrocarbons at Zawiya Oil Refinery in the Libyan Arab Republic. Matroud *et al*. (2021) isolated the fungi Macrophomina, *Aspergillus* sp. *A. niger*, *A. carbonarius*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Stemphylium* sp. and *Ulocladium* sp. from the soil in Al-Qadisiyah Governorate. Al-saadoon *et al*., (2022) [3] found 112 yeast isolates in different soil samples in the governorates of Basra and Dhi Qar, belonging to 16 types of ascomycota and 15 types of basidiomycota. And since hundreds of fungi and yeasts collapse and are lost every year, and many of them perish before they are identified, and because the insistence on moving forward on this bumpy road cannot be stopped by a deterrent, the aim of the research came to isolate and diagnose some types of fungi and yeasts present in the soils of the oil field refineries. for the governorates of Kirkuk and Erbil.

Materials and Methods
Collection of Filamentous Fungi and Yeasts
Twenty soil samples were obtained from the Bai Hassan oil fields and the K1 oil filling station of the governorates of Kirkuk and Erbil / Iraq.

Isolation of Filamentous Fungi and Yeasts
Filamentous fungi and yeasts were isolated from the soil according to the isolation method used in (Pan *et al*., 2009; Ihuma *et al*., 2022) using fungus-specific Potato Dextrose Agar (PDA) and Yeast Extract Malt Extract Medium (YM Agar). Fungi were purified using the single spore technique Rohilla (Salar, 2012).

Identification Tests of Fungi
It relied on to morphological characteristics of the cultured colonies represented by the color and diameter of the colonies, as well as the microscopic diagnosis, which was based on the shape of the fungal hyphae, the method of their branching, the presence of septa, the shape of the conidia, the number of conidia cells, the shape of the conidial carrier, the presence of stony bodies and other structures, as well as the observation of growth on the media The three basic cultures for diagnosis are: a growth test at a temperature of 25˚C and 37˚C on Zabek medium, yeast extract (CYA), medium (CY20S) supplemented with 20% sucrose, and barley extract medium (MEA) (Pitt and Hocking, 1997). The diagnosis was also made with the help of Prof. Dr. Nadim Ahmed Ramadan / Al-Hadbaa University College and based on the taxonomic keys by (Ellis, 1971; Pitt and Hocking, 2009) [12]. The fungal frequency percentage was calculated according to the following equation:
The percentage of fungal frequency = (samples in fungus appearing times number) / (total samples number) x 100 (Saleh *et al*., 2009)

Identification Tests for Yeasts

Morphological characteristics of colonies and microscopy

The isolates were cultured by planing method on Malt Extract Agar Medium (MEA) and incubated at 28 °C for 48 hours. Observations related to phenotypic traits were recorded and examined under a light microscope at 40X powers to note the shape of the yeast cells (Pitt and Hocking, 2009) [12].

Diazonium Blue B (DBB) Color test

This test was carried out according to the method of (Kurtzman and Fell, 1998) [9, 10].

Growth at 25° C and 37° C Test

Yeasts were cultured on solid media MEA by the method of streaking, and incubated at a temperature of 25 °C and 37 °C for a period of 3-7 days. The result is recorded as negative when there is no growth or positive when there is growth (Pitt and Hocking, 2009) [12].

Assessing ability to utilize nitrate as a sole nitrogen source

The test was conducted by transfer the part of culture by Streaking on the surface of Czapek Agar medium in petri dishes. Incubated in 28˚C for 3-7 days.

Assessing preservative resistance of glacial acetic acid

Inoculate petri dishes container Malt Acetic Acid (MAA) solidified with part of culture of the studied isolates by streaking and incubated in 28˚C for 3-7 days.

Growth at reduced water activities in high carbohydrate levels

Inoculate petri dishes container MY50G medium with part of each of the studied isolates by Streaking and incubated in 28˚C for 3-7 days.

Growth at reduced water activities and high level of sodium chloride

Planting isolation by streaking on MY10-12 medium incubated in 28˚C for 3-7 days (Pitt and Hocking, 2009) [12].

Mycelium formation test

Conducted the test to know the ability of yeasts on forming true mycelium and pseudo mycelium, By inoculating small flasks container 20 ml from Sabouraud's Glucose Broth Medium (SGB) with part of pure culture from yeasts, incubated the flask for 48 hours in 28˚C then examined the yeast growth under microscope at 40X, budding, cell shape and presence of mycelium and its shape whether true mycelium or pseudo mycelium (Kurtzman and Fell, 1998) [9, 10].

Diazonium Blue B (DBB) Color Test

The yeast grown on YM Agar medium and incubated in 28˚C for 10 days until the acus is formed in Ascomycetes. Added a drop to two drops from Diazonium Blue B reagent on the surface of the developing colonies and left to 2-3 minutes at laboratory temperature. The coloration of the colonies indicates that the dark red color, which is oblique to the violet, indicates that it belongs to the Bazidiomycetes, but if it coloration orange, it indicates that it belongs to Ascomycetes (Kurtzman and Fell, 1998) [9, 10].

Results and discussion

Isolation of yeasts

The growth of colonies growing on the media used for isolation showed the presence of 62 yeast isolates, as the highest number of isolates was 32 in the Bay Hassan field, and *R. mucilaginosa* was detected in the two isolation sites, 36.66% in the k1 oil filling station and 40.62% in the Bay Hassan field. While Pichia anomola was less frequent 10% and *G. candidum* 3.20%, respectively, the rest of the yeasts varied in their rates of appearance in the two isolation sites (Tables 1). These isolation results were consistent with many studies that indicate the presence and spread of yeasts in the soil widely (Sadaqni, 2000) [14], as the soil and water receive all or most of the nutrients from external sources such as plants, animals, and materials resulting from human activity, which are a food source for yeasts and fungi, and therefore The group of yeasts that are found in the soil changes in numbers and types according to the quantity and type of nutrients that reach it, but there are many types of yeasts that prevail in the soil permanently, and the yeasts isolated from the soil differ according to the type of soil and the percentage of moisture, as well as depending on the type of crop grown on the soil. (Pollock, 1992) [13]. Several studies indicated the possibility of isolating yeasts from the soil, as scientists were able to isolate 130 types of yeasts from the soils of different regions (Yorkov and Kemler, 2012) [18]. Sophie (2013) isolated the yeast *S. cerevisiae* from different types of soil in the city of Mosul. Ali and Khan, (2014) study of soil samples noted the presence of *S. rosinii*, *R. minuta*, *S. cerevisiae* and *S. exiguous*. Yaçin *et al*. (2018) was able to isolate 65 yeast species, most of which belong to *Rhodotorula* sp., *Candida* sp., *Yarrowia* sp., *Geotricum* sp., *Galactomyces* sp. and *Cytobasidium* sp. from oil contaminated soils. In addition, new types of black yeasts such as *Exophiala macquariensis* sp. in sub-Antarctic hydrocarbon-contaminated soils that are capable of degrading benzene, toluene, ethylbenzene, and xylenes (Zhang *et al*., 2019) [19].

Table 1: Types of yeasts and filamentous fungi isolated from the soil of K1 oil filling station and Bei Hassan field

Isolated Yeasts	Number of isolates	% Isolate	Isolation Site
R. mucilagenosa	11	36.66	K1 oil filling station
K. exigua	9	30	
Pichia anomola	3	10	
S. cerevisae	7	23.33	
Total	30	99.99%	
A. niger	9	56.25	
A. caespitosus	4	25	
A. fumigatus	1	6.25	
Penicillum spp.	2	12.5	
Total	16	100%	Total

Kloekera apiculata	3	9.37	Bei Hassan field
G. candidum	1	3.20	
Saccharomyces cerevisae	5	15.62	
Zygosaaccharomyces rouxi	2	6.25	
Rhodotorula mucilagenosa	13	40.62	
Kazachstania exigua	8	25	
Total	32	100%	
Penicillium spp.	6	9.37	
Aspergillus spp.	9	14.06	
A. nidulans	5	7.81	
A. Niger	15	23.43	
A. Parasiticus	10	15.62	
A. Caespitosus	7	10.93	
A. emericella quadrilieata	11	17.18	
Rhizoctonia spp.	1	1.56	
Total	64	99.96	Total

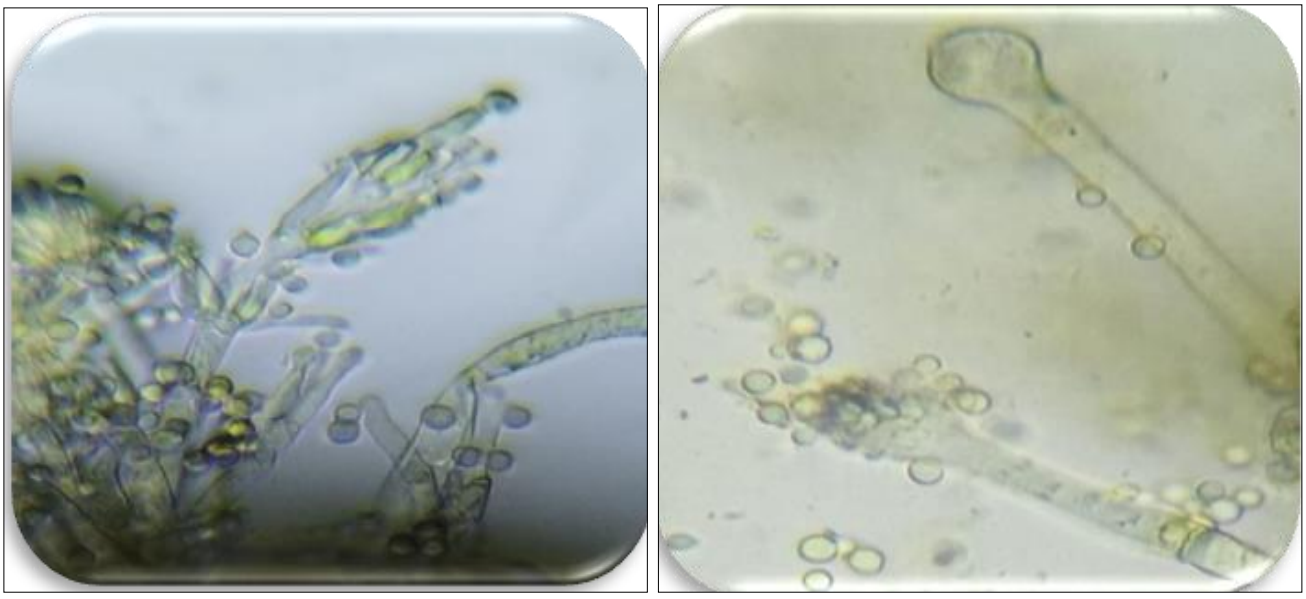
Isolation of Fungi

The isolation from the soils of the oil fields in the provinces of Kirkuk and Erbil showed that 80 isolates were obtained (Tables 1). The highest number of isolates was obtained from the soil of Bay Hassan field, as it reached 64 isolates, followed by k1 station (16) isolates. Our results agree with the findings of many studies that indicate the presence of fungi in different types of soil contaminated with crude oil and its derivatives, such as *A. ustus*, *P. lilacinium*, *A. nigr*, *P. ochrochloron*, *Trichoderma viride* fungi from crude oil and diesel contaminated soils (Essabri *et al*., 2019; Benguena and Chibani, 2021) [8, 5]. In another study conducted by Abdulla (2020), it was observed that there were 28 fungal isolates belonging to the genus Penicillium and 23 fungal isolates of *Aspergillus* sp. from soils contaminated with crude oil taken from the Al-Kasik and Qayyarah refineries.

Identification

Identification of Fungi

The results of the diagnosis of fungi showed the emergence of 9 different types from the soil of oil fields in the provinces of Kirkuk and Erbil, *A. niger* was the most frequent fungus in the two isolation sites, the number of isolates in (k1) station reached 9 isolates (56.25%) and 15 isolates (23.43%) in Bay field Well, the fungus *A. fumigatus* was the least frequent (6.25%) in K1 station. And *Rhizoctonia* spp. was less frequent in the field of Bei Hassan (1.56%), and the rest of the fungi varied in their appearance rates (Tables 1) Figure (1). These diagnostic findings are consistent with the description of each of them in the taxonomic keys (Ellis, 1971; Pitt and Hocking, 2009) [12].



E. quadrilineata *A. caespitosus*

Fig 1: Microscopic characteristics of fungi isolated on PDA medium

Identification of Yeasts

Cultural Characteristic

The results of the morphological characteristics of the yeast colonies growing on MEA medium, color, shape, diameter, nature of the edges of the colonies, height, texture, brilliance of the colonies and the shape of their surface, as well as the initial diagnosis (microscopic examination) indicated the presence of a number of isolates belonging to the same species, so the selection was made on 17 isolates (Table 2), and the initial diagnostic characteristics of these isolates matched the description of each of them by (Pitt and Hockind, 2009; Deàk, 2008; Kurtzman and Fell, 1998) [9, 10].

Table 2: Morphological characteristics of yeast colonies grown on MEA

Isolation	Morphological characteristics							
	Colony color	Colony shape	Colony diameter(mm)	Nature of colony edges	Colony height	Colony texture	Colony luster	Colony surface shape
S1	White	Circular	1.5	Irregular	Convex	Bowl	Opaque	Rough
S2	White	Irregular	5	Irregular	Flat	Membranous dermal	Opaque	Rough
S3	Creamy	Circular	2.6	Smooth r	Flat high center	Bowl	Opaque	Soft- smooth
S4	Creamy	Circular	1.3	Smooth	High	Bowl	shiny	Soft- smooth
S5	Creamy	Circular	2.6	Smooth	Convex	Bowl	shiny	Soft- smooth
S6	Orange	Circular	1.9	Smooth r	Convex	mucous	shiny	Soft- smooth
S7	White	Circular	4.2	Smooth	Convex	Bowl	shiny	Soft- smooth
S8	White	Irregular	7.8	Irregulars	Flat	Bowl	shiny	smooth

Microscopic examination

The results of the microscopic examination of the isolates showed a clear variation in the shape of the cells, from budding to spherical, oval or elongated, lemony, and some are sticky (Table 3) and of different sizes (Figure 3). These results were consistent with the specifications mentioned by (Fell and Kurtzman, 1998; Pitt and Hocking, 2009; Deák, 2008) [9, 10, 12, 7].

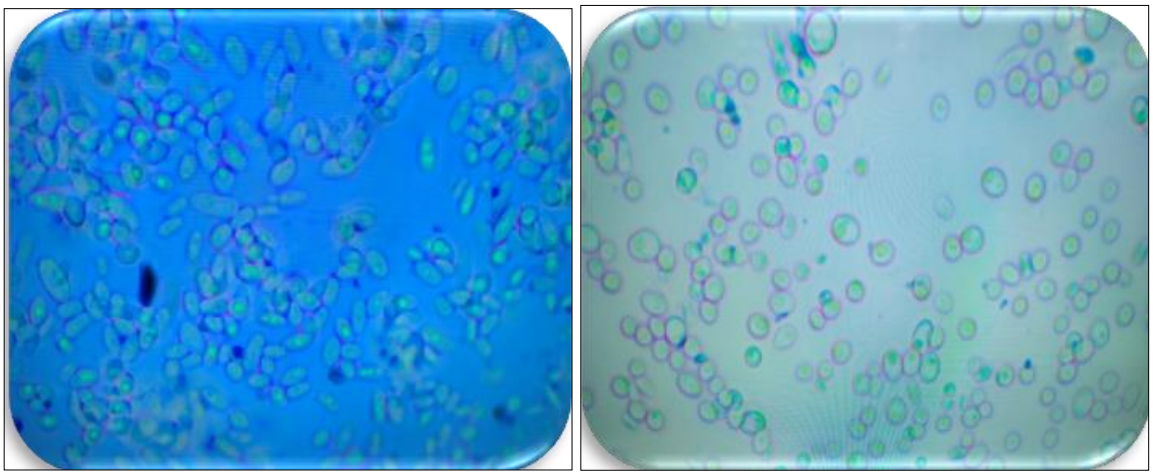
Mycelium Formation Ability Test

The results of the current study showed the ability of 6 isolates (75%) to form a false mycelium, while the results showed the ability of one isolate (S3 Geotrichum spp.) with a rate of 12.5% to form a true mycelium, and only one isolate, *Rhodotorula* spp., differed. S6 12.5% with its ability to give mycelium, as some of them formed true mycelium and others formed pseudomycelium, this characteristic is considered different among these types of yeasts, these results agreed with (Fell and Kurtzmsn, 1998; Deák, 1986). (Malla Obaeda, 2017), and based on the results obtained from culture traits, microscopic examination, and testing the ability of yeasts to form mycelium, it was found that the aforementioned isolates belong to the genera *Rhodotorula*, *Candida*, *Kodamaea*, *Kloeckera*, *Cryptococcus*, *Stephanoascus*, and *Saccharomyces* (Table 3).

Table 3: Microscopic examination of diagnosed yeasts isolates

Yeasts	Microscopic examination		
	Cell Shape	Cell Dimensions (µm)	Mycelium Formation Test
Pichia spp. S1	Spherical some are slightly elongated	3.5	-
Saccharomyces spp. S2	Spherical - small lemon	7	-
Geotrichum spp. S3	oval elongated (cylindrical)	4×6	+
Zygosaccharomyces spp. S4	Spherical slightly elongated	3.5	-
Kloeckera spp. S5	ovoid	6.5×10	-
Rhodotorula spp. S6	ovoid	15.6×8.3	+ / -
Kazachstania spp. S7	spherical	9.5	-
Candida spp. S8	oval elongated (cylindrical)	5×2.5	-

Spherical and lemon-shaped cells, the diameter of the cell was calculated, and for other shapes of cells, the length x width was calculated. (-): false mycelium, (+): true mycelium, (- / +): some form false mycelium and others form true mycelium.



S2 S3

Fig 3: Shapes of Isolated Yeasts Cells at 40X Magnification

Diazonium Blue B (DBB) Color Test

The results showed that among the 8 tested yeast isolates, only one isolate of *R. mucilaginosa* S6 (12.5%) was positive for this test, while the remaining 7 isolates were negative (87.5%). These characteristics are consistent with what was described by Kurtzman and Fill (1998) [9, 10]. Where he mentioned that this test is widely used in distinguishing between Ascomycetes and Bazidomycetes

Growth in 25˚C and 37˚C Test

The results of the ability to grow test showed that all yeast isolates (8) had the ability to grow 100% at a temperature of 25˚C, while the results showed the ability of 6 isolates (75%) to grow at 37˚C, as some of them grew weakly, 3 isolates (*G. candidum* S3, *Z. rouxii* S4, *R. mucilaginosa* S6) with a percentage of 37.5%, while two isolates with a percentage of 25% (*P.anomala* S1, *Klo. apiculata* S5) did not show a clear response to growth at this temperature. 2017) in his study of a group of yeast isolates at various temperatures, and the current results were similar to what he mentioned (Kirsop and Kortzman, 1988), a group of yeast isolates at various temperatures.

Assessing ability to utilize nitrate as a sole nitrogen source

It is noted from Table (4) that 5 isolates (62.5%) were able to benefit from nitrates as a sole source of nitrogen despite the varying ability to benefit from it, while the remaining 3 isolates (37.5%) were negative for the test, and this is consistent with what was reached by (Malla Obaida, 2020; Ebabhi *et al*., 2013).

Assessing preservative resistance of glacial acetic acid

The results of this test came in table (4) to show that 3 isolates (25%) were positive for this test, while the remaining 5 isolates (62.5%) showed a negative result for this test, and this is consistent with what was mentioned by (Pitt and Hocking, 2009) [12].

Growth at reduced water activities in high carbohydrate levels

The current results revealed as Table (4) that 4 isolates (50%) were negative for the test, while the remaining 4 isolates (50%) were positive for the test, and this agrees with what was mentioned by (Pitt and Hocking, 2009) [12].

Growth at reduced water activities and high level of sodium chloride

The results shown in Table (4) showed that 5 isolates (62.5%) were negative for the test, while three isolates (37.5%) were positive for the test, and this result was close to what was mentioned (Pitt and Hockin, 2009) [12].

Table 4: Biochemical diagnostic tests for the yeasts isolates under study

Diagnosed Yeasts	Test Type						DBB Test*
	Ability to grow in						
	25˚C	37˚C	Presence of nitrate as a source (N)	Presence of glacial acetic acid	Low water level high carbohydrate	Low and high water level of NaCl	
P. anomala S1	+++	-	+++	-	+	-	▫
S. cerevisiae S2	+++	+++	-	++	-	-	▫
G. candidum S3	+++	+	+++	-	++	+	▫
Z. rouxii S4	+++	+	-	-	+++	+++	▫
Klo. apiculata S5	+++	-	+	-	-	-	▫
R. mucilaginosa S6	+++	+	++	-	-	-	▪
K. exigua S7	+++	+++	-	-	-	-	▫
C. utilis S8	+++	+++		+++	+	+	▫

(-): no growth, (+): weak growth, (++): medium growth, (+++): good, dense growth :(▪) ,Basidian yeasts, (▫): cystic yeasts

*DBB= Diazonium Blue B.

Conclusion

- Below are the most important conclusions of the current study
- The isolation results showed that the local environment (soil) is a rich source of different types of yeasts and filamentous fungi
- The isolates showed great variation in the phenotypic traits of the colonies.

References

1. Abu-Mejdad NM, Al-Badran AI, Al-Saadoon AH. A new record of interesting basidiomycetous yeasts from soil in Basrah province/Iraq. Basrah Journal of Science,2019:37(3):307-334.
2. Al-Khafaji, Zahra Mahmoud, Sanaa Burhan Al-Din Al-Nama, Al-Khafaji and Shatha Salman Al-Azzawi. A Study of Common Yeasts of Baghdad Region 2: Cereal Products and Dry Legumes, Iraqi Journal of Microbiology,1992:4 (1-2):54-60.
3. Al-Saadoon AH, Ali NMJ, Ali LH, Al-Badran AI. Effect of Some Ecological Factors on Occurrence of Yeasts in Soil and Sediment from Iraq. Biomedicine and Chemical Sciences,2022:1(3):117-125.
4. Assis SMP, Mariano RLR. Antagonism of yeasts to *Xanthomonas campestris* pv. Campestris on cabbage phylloplane in field. Rev. Microbiol,1999:30:191-195.
5. Benguenab A, Chibani A. Biodegradation of petroleum hydrocarbons by filamentous fungi (Aspergillus ustus and Purpureocillium lilacinum) isolated from used engine oil contaminated soil. Acta Ecologica Sinica,2021:41(5):416-423.
6. Brisha Jaber Zayed. Yeasts: their production techniques and their role in industry, food spoilage and human health (first edition). King Saud University, Riyadh, 2010, 493.
7. Deák T. Handbook of Food Spoilage Yeasts (second Edition). CRC Prees Taylor and Francis Group. Boca Raton London New York, 2008.
8. Essabri AM, Aydinlik NP, Williams NE. Bioaugmentation and biostimulation of total petroleum hydrocarbon degradation in a petroleum-contaminated soil with fungi isolated from olive oil effluent. Water, Air, & Soil Pollution,2019:230:1-16.

9. Kirsop BE, Kurtzman CP. Living resources for biotechnology " Yeasts " Cambridge Univ. Press. Cambridge, 1988.
10. Kurtzman CP, Fell JW. The yeasts, A Taxonomic Study.4th ed., Elsevier Science B.V.,The Netherland, 1998.
11. Pa LX, Yang DF, Shao L, Li W, Chen GG, Liang ZQ. Isolation of the oleaginous yeasts from the soil and studies of their lipid-producing capacities. Food Technol. Biotechnol.,2009:47(2):215-220.
12. Pitt JI, Hocking AD. Fungi And Food Spoilage. Springer Dordrecht Heidelbery London New York, 2009.
13. Pollock TJ. Pullulan from polymorphic Aureobasidium pullulans SIM Ind. Microbial. News.,1992:42:147-155.
14. Sadaqni GS. A contribution to the identification of yeasts spread on some Syrian fruits. Damascus University Journal of Agricultural Sciences,2000:16(2):79-89.
15. Suad Muhammad Khalifa Abu Al-Ghaith, Ahlam Al-Qamudi Muhammad Zaait. Isolation, identification and testing of the efficiency of some fungi in hydrocarbon degradation from oil-contaminated soil. Scientific Journal of Applied Sciences of Sabratha University, 2020, 78-90.
16. Willemsen M, Breynaert J, Lauwers S. Comparison of auxacolor with API 20 C Aux in yeast identification. Clinical Microbiology and Infection,1997:3(3) 369-375.
17. Yalçın HT, Ergin-Tepebaşı G, Uyar E. Isolation and molecular characterization of biosurfactant producing yeasts from the soil samples contaminated with petroleum derivatives. Journal of basic microbiology,2018:58(9):782-792.
18. Yurkov AM, Kemler M, Begero D. Assessment of yeast diversity in soils under different management regimes. fungal ecology,2012:5(1):24-35.
19. Zhang C, Sirijovski N, Adler L, Ferrari BC. Exophiala macquariensis sp. nov., a cold adapted black yeast species recovered from a hydrocarbon contaminated sub-Antarctic soil. Fungal biology,2019:123(2):151-158.
20. Akhtar R, Showkat S, Saini N. "Isolation and Identification of soil born fungi from agricultural fields of Dehradun, India". International Journal of Ecology and Environmental Sciences,2022:4(3):60-65.